

Effect of Neomycin and Vancomycin on Growth of Some Rumen Bacteria¹

R. B. COMFORT² AND H. S. RAGHEB

Department of Biochemistry, Purdue University, Lafayette, Indiana 47907

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Most observations of the effect of antibiotics on the rumen microbial population were casual observations subsequent to incorporation of antibiotics in the animal feed, and only a few studies (2) were conducted in vitro. S. M. Finegold, L. A. Siewart, and W. L. Hewitt, (Bacteriol. Proc., p. 59, 1957) devised a medium containing neomycin (100 to 200 $\mu\text{g/ml}$) and vancomycin (7.5 $\mu\text{g/ml}$) to isolate anaerobic *Bacteroides* from the human oral cavity. We, therefore, attempted to study the effect of these antibiotics on rumen microorganisms with the objective of selective isolation of rumen *Bacteroides* bacteria.

Pure cultures which represent important cellulolytic or amylolytic bacteria in the rumen were grown anaerobically in 8-ml quantities of the general-purpose medium (1). This medium contained 0.1% (w/v) glucose, 0.1% (w/v) cellobiose, 0.2% (w/v) cellulose (Whatman no. 1 slurry), 0.5% Trypticase (BBL), minerals, 0.1% (v/v) of each of isovaleric, *n*-valeric, isobutyric, and 2-methyl butyric acids, carbonate-bicarbonate buffer, and cysteine hydrochloride. To each tube was added 1 ml of the sterile antibiotic solution and 1.0 ml of the inoculum. In control tubes, the antibiotic solution was replaced by 1.0 ml of sterile water. Inocula, at an optical density between 0.6 to 1.0, were taken from cultures that had been grown in the same medium for 18 hr. Growth was measured by determination of optical density both initially and after incubation for 18 hr at 39 C. Viable counts were made by diluting each inoculum in buffered mineral solution containing cysteine hydrochloride (1) and then inoculating culture tubes, each containing 9 ml of the general-purpose medium plus 2% agar. When neomycin was added, its final concentration was 100 $\mu\text{g/ml}$. The roll-tube technique (3) was used for inoculation. The tubes were incubated for 10 days at 39 C and the colonies were counted.

The growth of cultures, as determined by optical density (Fig. 1), generally decreased as the concentration of neomycin increased to 20 $\mu\text{g/ml}$. *Ruminococcus flavefaciens* was the least affected, whereas *Butyrivibrio fibrisolvens* was the most inhibited. Little changes in optical density occurred thereafter at higher concentrations of neomycin. This antibiotic effect was better observed by viable-counts determination (Table 1). Neomycin, at a concentration of 100 $\mu\text{g/ml}$, showed no significant effect on viable counts of *Bacteroides succinogenes* S85 and *R. flavefaciens* C94, but growth of *B. fibrisolvens* D1 and *Bacteroides rumenicola* 23 was severely depressed. Vancomycin, at a concentration of 7.5 $\mu\text{g/ml}$, completely inhibited all cultures examined. The

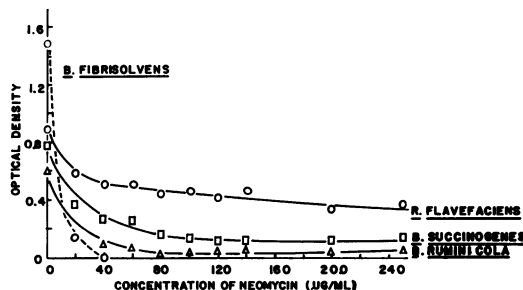


FIG. 1 Effect of neomycin on growth of pure cultures of rumen bacteria.

lack of growth inhibition of *B. succinogenes* by neomycin agrees with the results of Finegold et al. (Bacteriol. Proc., p. 59, 1957). However, in our studies, another rumen *Bacteroides*, *B. rumenicola* 23, was inhibited by this antibiotic. In regard to vancomycin, it cannot be established whether its effect is bacteriostatic or bactericidal, since viable counts were not made. If one would extrapolate these results for selective isolation of *Bacteroides* from the rumen, keeping in mind that many other factors in the rumen may affect results obtained with pure cultures, incorporation of neomycin in the media does not appear to be promising. Other organisms present in the rumen in large numbers may survive in the presence of this antibiotic. Indeed, viable counts for

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² Present address: Department of Microbiology, H. J. Heinz Co., Pittsburgh, Pa.

TABLE 1. *Effect of neomycin on viable counts of pure cultures of rumen bacteria*^a

| Organism | No. of bacteria per ml ^a | |
|----------------------------|-------------------------------------|----------------------|
| | Control | Neomycin (100 µg/ml) |
| <i>B. succinogenes</i> S85 | 60×10^7 | 80×10^7 |
| <i>B. fibrisolvens</i> D1 | 61×10^6 | <10 |
| <i>B. ruminicola</i> 23 | 17×10^7 | 9×10^2 |
| <i>R. flavefaciens</i> C94 | 61×10^8 | 22×10^8 |

^a Roll-tube technique employed.^b Mean of five determinations.

R. flavefaciens in the present study indicated no inhibition in the presence of neomycin. Since the

effect of antibiotics upon the gastrointestinal flora of animals is of public health concern, further studies of these effects on rumen bacteria are necessary.

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